

Review Article

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## Anestrous in Buffaloes and Different Treatment Regimens: A Mini Review

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### ABSTRACT

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Buffalo is regarded as Black gold and more than 50 per cent of the rural poor in India are dependent on livestock for their livelihood. The buffaloes have inherent problems of summer anestrous, poor expression of estrus and therefore prolonged calving to conception interval. The advent of ultrasonography has aided in better understanding of follicular wave pattern during cyclic and non-cyclic stages of reproductive cycle. The present review summarises various aspects of buffalo reproduction including Follicular dynamics, anestrous and different treatment regimens.

### Introduction

India has vast resource of livestock, which plays a vital role in improving the socio-economic condition of rural masses. India ranks 1<sup>st</sup> in respect of buffalo, 2<sup>nd</sup> in cattle and goat and 3<sup>rd</sup> in sheep population in the world (Annual Report, Department of Animal Husbandry and Dairying, 2007-08). The country is highest milk producer of the world with annual milk production of 101.9 million

tons (Anon, 2007). The milk is contributed mainly by buffaloes (55.5%) followed by cattle (40.0%) and goat (4.5%), respectively. The proportionate contribution of livestock sector is Rs 984 billion (5.51%) of total Gross Domestic Product (DairyIndia, 2007).

The water buffalo domesticated since 5000 B.C. has attained important place in Indian economy. Considering the importance of buffaloes and its production potential, the

buffalo has been termed as —The Black Gold (Acharya and Bhat, 1988). About 50 per cent of the rural poor in India are dependent on livestock for their livelihood (Thornton *et al.*, 2002). The high yielding Murrah is the Holstein-Friesian of the buffalo world. Buffalo milk contains about twice as much butterfat as cow milk. Buffaloes are also valued for meat and draught purpose (Bandyopadhyay *et al.*, 2003). In subtropical countries like India, the vagaries of climate like extreme hot humid environment with hot winds adversely affect the performance of buffaloes. The high ambient temperature during summer enhances level of prolactin which is anti-gonadotrophic (Kaker *et al.*, 1981), thus affecting the hypothalamic-pituitary-ovarian pathway resulting in acyclicity (Short *et al.*, 1990). Basic reproductive physiology, with regard to regulation of ovarian functions pertaining to folliculogenesis, steroidogenesis and corpus luteum formation, structure, function and regression, has been studied more extensively in female cattle than in buffaloes.

Understanding the mechanisms of folliculogenesis and follicular turnover during estrous cycle are essential for solving the problems of low reproductive efficiency in buffaloes. During late 1980<sup>s</sup>, application of transrectal ultrasonography for imaging ovarian follicles opened up new vistas for studying folliculogenesis in cattle and buffaloes. Ovaries are the primary organs of reproductive system, which undergo conspicuous structural changes during different stages of estrous cycle as a result of the growth and regression of follicles and corpus luteum (CL). Ultrasonography is relatively simple, effective and safe both to subject and operator, portable and ultra-rapid since the sonographic imaging facilitates immediate interpretation and diagnosis in most circumstances (Rajamahendran *et al.*, 1994). Real time ultrasonic imaging provides

a noninvasive and non-disruptive technique to directly examine external and internal anatomy of reproductive organs *in situ* and to monitor closely the dynamic reproductive events e.g. follicular, luteal growth and regression, pregnancy, transition of uterus from a diestrus to anestrus stage (Griffin and Ginther, 1992). Ultrasonography has facilitated immensely in better understanding of certain poorly understood areas of the reproduction (ovarian follicular dynamics, corpus luteal functions and pregnancy establishment). Infertility among dairy animals continues to be a major bottleneck in exploiting the fullest production potential of our animal wealth. An annual loss due to reproductive inefficiency is more than 500 crore rupees (Dairy India, 2007). Reproductive efficiency is the key determinant influencing productivity of the livestock and is adversely affected by late attainment of puberty, silent estrus, variability of estrous length, seasonality of calving, dystocia, genital prolapse, retention of fetal membranes, long postpartum anestrus and subsequent calving interval (Barile, 2005). Low reproductive efficiency in the buffalo remains a major economic problem globally and its incidence is higher in India (Kumar *et al.*, 2009). Prolonged postpartum acyclicity and anestrus are major causes of economic loss to buffalo breeders (El-Wishy, 2007). In India, incidence of anestrus in buffaloes has been reported from 25 to 67% (Singh *et al.*, 2003; Pandit, 2004). Clinical survey revealed higher incidence of anestrus and inactive ovaries in buffaloes than in cows in India (Nagaraju *et al.*, 1991). The percentage of anestrus is more in rural areas mainly due to malnutrition and improper managerial practices (Kumar and Sharma, 1991).

Attempts have been made by various researchers to induce cyclicity in anestrus buffaloes during peak breeding and low breeding periods with the help of hormonal

and non-hormonal preparations (Lakra *et al.*, 2002; Singh, 2003). Favorable effects of GnRH and its analogues in inducing early postpartum ovarian activity and estrus have been reported in buffaloes (Saini and Lohan, 2003). Progestogens have widely been used for induction of estrus and ovulation in acyclic animals. Hormonal preparations including PRID (Shah *et al.*, 1987); Synchronate-B (Misra *et al.*, 2003); Crestar, (Malik *et al.*, 2003; Malik, 2005); Controlled Internal Drug Release followed by PMSG (Ravikumar *et al.*, 2005; Singh, 2004) have been employed in the treatment of anestrus buffaloes. Duraprogen plus Progynon depot followed by PMSG have also been observed to provide encouraging results during breeding season and low breeding season (Singh, 2004). Recently, a novel protocol, Ovsynch (GnRH-PGF<sub>2</sub>α-GnRH regimen) was developed by Pursley *et al.* (1995) to synchronize ovulations in lactating dairy cows was also tried in buffaloes (De Rensis, *et al.*, 2005; Malik, 2005) with variable degrees of success. The precise synchrony allows for successful fixed time A.I. without the need for detection of estrus and has been applied widely in cattle breeding. However, the comparative efficacy of these hormonal regimens of Ovsynch plus CIDR and Ovsynch plus progesterone in Murrah buffaloes during summer season has not been attempted.

Buffaloes hold immense socioeconomic importance to marginal farmers in several developing countries. The success of dairy cattle and buffalo husbandry lies in ensuring proper and optimal reproductive rhythm of each individual female in the herd. India has largest buffalo population (97 million) which account for 94.5-million-ton milk production (Dairy India 2007). Despite greater contribution in terms of milk production in relation to less population as compared to cattle, per head production of buffalo still

remain low because of delayed puberty, higher age at first calving, longer inter calving interval, problem of heat detection, low conception rate, high stress of lactation and variation in conception during different months of year (Jainudeen *et al.*, 2000). Anestrus in Indian buffalo due to smooth ovaries is the single largest cause of infertility (Parkinson, 2001). It is the most serious reproductive disorder of Indian buffaloes which affects farmer's economy by reducing calf crop and milk production. Out of various etiological factors like nutritional, lactational, stress etc. Malnutrition is the most common cause of anestrus under field condition. Sreemannarayana and Rao (1996) observed incidence of anestrus as high as 67% and 61% in rural cattle and buffaloes, respectively.

Postpartum anestrus in buffaloes is responsible for long calving intervals (Borghese *et al.*, 1993) which is usually reported with higher frequency in primiparous and ageing buffaloes especially when the parturient period coincides with increasing daylight hours (Zicarelli, 1997), thereby, indicating a seasonal trend in reproduction (Campo *et al.*, 2002). In addition to acyclicity, female buffaloes also experience weak estrus signs at irregular intervals with a propensity to develop ovarian pathology (Esposito *et al.*, 1992). Estrus synchronization protocols, largely derived from cattle have yielded variable results in buffalo (Barile *et al.*, 1997). Although failure of timed ovulation in synchronized buffaloes has been suggested as an important cause of poor fertility (Baruselli, 2001), it has not been studied critically. There are some research reports of ovarian follicular dynamics in buffalo (Manik *et al.*, 1994; Taneja *et al.*, 1996; Baruselli *et al.*, 1997) but a critical comparison of the effects of age and parity on ovarian follicular dynamics and hormonal profiles has not been studied. A comprehension of the pattern of ovarian follicle recruitment and selection in

postpartum buffaloes can provide required insight and lead to refinement of protocols for synchronization/induction of ovulation for successful artificial insemination. Conception rates after fixed time A.I. following estrus synchronization are considerably lower than those for A.I. at detected estrus, because of more variable interval to ovulation after PGF<sub>2</sub> injection (Pursley *et al.*, 1997a). Reduced fertility following these synchronization protocols made it necessary to understand ovarian follicular and CL dynamics in cattle. Synchronization of ovulation and fixed time artificial insemination (FTAI) is a promising protocol and has the potential to enhance pregnancy rates and success of A.I. programme. Ovsynch is one such estrus and ovulation synchronization protocol which has been used in lactating cattle for FTAI, stimulating ovarian follicle growth, maturation and ovulation (Pursley *et al.*, 1995). Recently, success of Ovsynch was reportedly associated with pretreatment ovarian picture (Rohilla, 2003) and accordingly a modified protocol Ovsynch -Plus 'was tried in buffalo heifers (Singh *et al.*, 2004). However, its application in postpartum buffaloes is yet to be assessed. Progestogens have also been widely used for induction of estrus and ovulation in acyclic animals. Several researchers (Patel *et al.*, 2003; Misra *et al.*, 2003) used different progesterone preparations and got varied degrees of success. In buffaloes, Lohan *et al.*, (2001) found that estrus induced with Crestar+PMSG were mostly anovulatory leading to poor fertility. In low breeding season, Crestar was not suitable for synchronization of estrus and ovulation (Bartolomeu *et al.*, 2002).

The present review is an attempt to provide details of research carried out so far on various aspects of ovarian follicular dynamics during early postpartum period and anestrus condition as well as on estrus induction and

synchronization particularly in buffaloes.

### **Buffalo reproduction**

Despite several recent advances in the field of animal reproduction, infertility as manifested by true anestrus remains a major economic problem in the cattle and buffalo in most tropical countries. A long calving interval in the buffalo is mainly due to delay in resumption of ovarian cyclicity during early post-partum period.

### **Puberty**

As compared to cattle, puberty is delayed in buffalo (Jainudeen and Hafez, 1993). The delay in puberty and consequent delay in conception is one of the main causes of low reproductive efficiency in buffalo, thus lengthening nonproductive life. Studies across the world indicate that puberty is significantly affected by breed, season, climate, feeding systems and growth rate (Borghese *et al.*, 1994).

### **Seasonality**

Although buffaloes are polyestrous, their reproductive efficiency shows wide variation throughout the year. Season of calving also influences onset of postpartum ovarian activity (Jainudeen *et al.*, 1983), as buffaloes calving in summer resume ovarian activity earlier than those calving in winter (Lundstrom *et al.*, 1982). Climate, and particularly photoperiod (by melatonin secretion), plays a pivotal role in seasonality of estrus in buffaloes (Parmeggiani *et al.*, 1993). The proportion of buffaloes exhibiting estrus during the period of short-day length is significantly greater than during the period of long day length, indicating that decreasing day light is a stronger determinant of the resumption of ovarian activity (Barile, 2005). Effect of heat stress has been observed on

induced estrus also which was evident when same treatment in winter induced 100 % cyclicity with 100 % conception rate as compared to 72 % cyclicity and 50 % conception rate and more variable and silent estrus in summer (Markandeya *et al.*, 1993).

### **Estrus and estrous cycle**

Seasonal variation in duration of estrus has been reported in buffaloes with 14, 18 and 8 to 10 h, respectively, during monsoon, winter and summer seasons (Janakiraman, 1978). The average duration of estrus in buffaloes has been reported to be 18 to 20 h with a range of 6 to 49 h (Gill *et al.*, 1973). The interval from end of estrus to ovulation has been observed to be 11 h (range 5 to 24 h) in Indian buffaloes (Luktuke and Ahuja, 1961). Buffaloes exhibited bellowing, frequent urination, restlessness, swollen vulva and mucus discharge as important estrus symptoms (Singh *et al.*, 1984). Silent estrus (ovulation unaccompanied by estrus) is a major problem in buffalo breeding (Jainudeen, 1984). Use of vasectomized teaser bull has solved the problem of estrus detection to some extent, but it is laborious and time-consuming process.

### **Ultrasonographic monitoring**

Transrectal ultrasonic imaging provided a means for repeated, direct, non - invasive monitoring and measuring of follicles regardless of their depth within the ovary (Johnson *et al.*, 1994; Garcia *et al.*, 1999). The technique has been used to diagnose pregnancy, fetal number and viability in small ruminants for the last several years (Buckrell, 1988).

Ultrasonography utilizes high frequency sound waves to produce images of tissues and internal organs. The sound waves are produced by vibrations of specialized crystals

called —piezoelectric crystal housed in the ultrasound transducer. Pulse of electric current produces the vibrations of these crystals and high frequency sound waves are generated. By positioning of transducer, the sound waves are directed at the target tissue for reflection which are called echoes. These echoes are inaudible to human ear. The proportion of sound waves that is reflected or echoedis received by the same —piezoelectric crystals converted into electrical impulses and displayed on the ultrasound screen/monitor as a series of grey dots extending from black to white. These sound waves produce echoes that vary in intensity depending on the density of reflecting surface. Liquid do not reflect sound wave, therefore, the image of a liquid containing structure is non -echogenic and appears black on the screen, e.g. ovarian follicular fluid, cystic fluid, embryonic vesicle etc. Dense tissue strongly reflects sound waves and appears white on the screen and being termed as hyper-echogenic structures e.g. fetal bones, pelvic bones, cervix etc. Soft tissues reflect sound waves and produce image of varying grey shades and called echogenic e.g. corpus luteum, endometrium etc.

Ultrasonography has proved to be an effective means of monitoring and evaluating ovarian follicles in bovines (Pierson and Ginther, 1988). In heifers, ultrasonographic monitoring of individual follicles has been used to study the temporal association between FSH surges and initiation of follicular wave (Adams *et al.*, 1992). Taneja *et al.*, (1996) monitored effect of the presence of dominant follicle (DF) and concluded that superovulatory response can be improved by ultrasonographic observation of status of follicular dominance prior to treatment. Silcox *et al.*, (1993) demonstrated that the ability of dominant follicles to respond to exogenous GnRH is dependent on the stage of development at the time of treatment. The phases of dominant follicle



were determined by daily ultrasonography. Similarly, stage of estrous cycle at which synchronization treatment is initiated influences reproductive response of FTAI (Ovsynch protocol) as it affects follicular development and plasma progesterone profiles (Moreira *et al.*, 2001).

In contrast to the other techniques, transrectal real time ultrasonographic screening of animal 's reproductive tract reveals true images of structures which are not possible, otherwise even by highly skilled palpators per rectally (Reeves *et al.*, 1984). Pieterse *et al.*, (1990) compared ultrasonography with rectal palpation and found that for detecting follicles, ultrasonography was a significantly better method than rectal palpation. Application of transrectal real time ultrasonography as a research tool to study bovine reproduction represents a technological breakthrough that has revolutionized our understanding of the reproductive biology. Ultrasonography has been found very useful in identifying CL in the ovary. Ribadu *et al.*, (1994) reported an accuracy of 95.7 % with ultrasonography whereas it was about 85 % with rectal palpation in detecting a CL. Ultrasonography, coupled with hormone estimation, gives the most reliable evaluation of ovarian activity (Ribadu *et al.*, 1994).

### **Anestrous and its treatment**

Anestrus is classified as either preservice or post-service (Zemjanis, 1980). Preservice anestrus is defined as the absence of observed estrus in the immediate postpartum period. Post-service anestrus is defined as absence of observed estrus following an unsuccessful insemination. The possible underlying reasons for anestrus could be physio-pathological in origin or they may be due to suckling, nutrition, infections of reproductive tract etc.

Various hormonal preparations used for induction of estrus in anestrus buffaloes range from the use of synthetic strogen (stilbestrol), estradiol as valerate or benzoate, gonadotrophins or analogues (Singh and Singh, 1986; Aminudeen, 1991). A variety of progestational compounds have been administered in different forms (Shankar *et al.*, 1996; Kundu, 1998; Malik, 2005) to mimic the luteal function by blocking the release of gonadotrophins from pituitary, so that subsequent withdrawal of these compounds may result in release of gonadotrophins to initiate follicular activity in ovaries with establishment of estrous cycles

### **Progestogens**

Progestogens have been widely used for induction of estrus and ovulation in acyclic animals. Various progesterone compounds have been administered to mimic the luteal function by blocking the release of gonadotrophins from pituitary, so that the subsequent withdrawal of these compounds may result in release of gonadotrophins to initiate follicular activity in ovaries with establishment of estrous cycles (Peters, 1986). Various progesterone preparations including melengesterol acetate (MGA; Kumar, 1999), progesterone releasing intravaginal device (PRID; Shah *et al.*, 1987), Crestar (Lohan *et al.*, 2001; Malik *et al.*, 2003), Syncromate-B (Zahid *et al.*, 2003; Misra *et al.*, 2003); progesterone and PMSG alone or in combination (Randhawa *et al.*, 2003) have been employed in the treatment of postpartum anestrus buffaloes but with wide variation in induction of cyclicity and subsequent conception rates.

### **Ear implants**

Auricular implants of progestogen (Crestar® or Syncromate-B) have been used to synchronize estrus and ovulation. These implants contain

norgestomet (17 $\alpha$ - acetoxy-11 $\beta$ -methyl-19-norpreg-4-en-3,20dione) which is more potent than natural progesterone and therefore, lower doses are required. Therefore, hydron implant Syncromate-B presents 6 mg of norgestomet, while silicone implant Crestar<sup>®</sup> presents 3 mg of norgestomet. Silicone implant causes progesterone like compounds to be released in a more homogenous and linear way, while hydron implant releases norgestomet in high quantities in the first two days and the quantity decreases during the following days (Kesler *et al.*, 1997). Crestar<sup>®</sup>+eCG protocol has been tried for induction of cyclicity in postpartum buffaloes and heifers by various researchers with varying success (Younis *et al.*, 1996; Lohan *et al.*, 2001; Pant *et al.*, 2002; Dahiya *et al.*, 2003; Pateletal, 2003 and Singh *et al.*, 2004; Malik, 2005).

Upon removal of the implant after 9-10 days, most buffaloes (>50%), return to estrus within 3-5 days with first service conception rates ranging from 40 -60 % (Singh *et al.*, 1988). If prostaglandin is administered at implant removal, it causes regression of CL and better synchrony (Pargaonkar *et al.*, 1988). In buffaloes a 40 % conception rate has been recorded following FTAI after Syncromate -B treatment (Singh *et al.*, 1983). With this treatment, cycling females may have higher pregnancy rates than acyclic animals (Sanchez *et al.*, 1995), since CL effectively modulates gonadotrophin secretion.

Zicarelli *et al.*, (1997) studied the use of Crestar<sup>®</sup> and PRID in milking buffaloes under field conditions and demonstrated low efficiency of treatments with pregnancy rates varying from 14.4 to 29.2 % for both groups, respectively. Administration of 500 -1000 IU of PMSG at the time of implant removal improves the chances of conception in buffaloes (Singh *et al.*, 1988; Younis *et al.*, 1996; Malik, 2005).

### **Melengesterol acetate (MGA)**

Feeding MGA @ 0.5-1.0 mg/animal/day for 14-17 days has also been used as a source of progesterone to induce estrus in buffaloes (Shukla *et al.*, 1972). Although cost of treatment is low and means of administration is easy but lower fertility was observed, which may be due to increased number of atretic follicles, failure of follicles to ovulate, reduced size of CL, reduced sperm transport and cleavage rate (Odde, 1990). However, some trials reported better results when prostaglandin was administered 16-18 days after MGA (Rajesh, 1999).

### **Progesterone releasing intravaginal devices**

Progesterone is administered via the intravaginal route by means of intravaginal devices. Initially, sponges were used which posed problem of retention. This led to development of silastic coils (Roche, 1976) and silicon rubber implants (Ellicot *et al.*, 1977) impregnated with progesterone and finally to PRID and CIDR (Welch, 1985). These have not only better retention property but also release progesterone at a controlled rate. MacMillan *et al.*, (1985) found that CIDR-B had better retention property than any other intravaginal device and since then it is the most commonly used intravaginal device. These are used alone or in combination with other hormonal drugs. Incorporation of estradiol benzoate (EB) as a luteolytic agent has enabled short-term PRID/CIDR treatments to synchronize estrus effectively (Singh *et al.*, 1988; Subramaniam and Devrajan, 1991). Using PRID alone for synchronizing estrus in buffaloes gave poor results (Saini *et al.*, 1986), attributed to relatively high incidence of anovulatory estrus. Subramaniam and Devrajan (1991) observed better estrus and conception rates when prostaglandin was administered on the day of CIDR removal than those treated with

CIDR alone. Bartolomeu *et al.*, (2001) studied the effect of fixed-time artificial insemination in Murrah buffaloes after synchronizing them with CIDR/EB or CIDR/GnRH and got the conception rate of 22.8 percent and 26.7 percent respectively. Murugavel *et al* (2009) recorded ovulation rate (81%, 47.4%) and pregnancy rates (38.1%, 21.1%) in eCG+CIDR and CIDR treated non -cyclic buffaloes, respectively.

### **GnRH and PGF<sub>2α</sub> combinations**

GnRH has been used to induce LH release and ovulatory cycle in postpartum anestrous buffaloes (Nasr *et al.*, 1983). The GnRH-PGF<sub>2α</sub> protocol involves treating animals with GnRH (day 0) followed by an injection of PGF<sub>2α</sub> on day six for luteolysis. This technique eliminates the need for estrus detection for the six- or seven-days period preceding the PGF<sub>2α</sub> treatment and enables the synchronized estrus in approximately 80 % females, during a period of less than four days following PGF<sub>2α</sub> induced luteolysis (Twargiramungu *et al.*, 1994). Fertility rates in GnRH-PGF<sub>2α</sub> treated cows inseminated at detected estrus varied between 65 and 85% and were identical to those of cows treated with PGF<sub>2α</sub> only (Twargiramungu *et al.*, 1992). Neglia *et al.*, (2003) observed a pregnancy rate of 45 % in buffalo cows synchronized with PGF<sub>2α</sub> alone and 48.8 % when PGF<sub>2α</sub> was combined with GnRH injection at AI.

### **GnRH-PGF<sub>2α</sub>-GnRH (“Ovsynch”)**

A novel protocol for ovulation synchronization named “Ovsynch” was developed for cattle, which makes use of GnRH-PG-GnRH injections (Pursley *et al.*, 1995). This protocol controls follicular development and life span of the CL. The precision of estrus and high fertility rates are due to the GnRH luteinizing or ovulating the

mature follicle and initiating recruitment and selection of a new dominant follicle. An advantage of this regimen is that it can be used at any stage of the estrous cycle and eliminates the use of progestogen besides promoting the resumption of ovarian activity in acyclic postpartum animals. The use of ovulation synchronization with FTAI in buffaloes provides advantages, similar to those found in cattle and additionally by foregoing the need for estrus detection, which is rather difficult laborious and time consuming (Baruselli *et al.*, 1994).

Follicular wave emergence and luteal regression, with GnRH and PGF<sub>2α</sub> administered 7 days apart, has been practiced with widespread acceptance in dairy herd reproductive management. A second dose of GnRH given 48 h after PGF<sub>2α</sub> in Ovsynch program, induces ovulation of the dominant follicle and allows FTAI 16 h after GnRH (Pursley *et al.*, 1995). When AI was done at fixed time after GnRH- PGF<sub>2α</sub>-GnRH programme, the pregnancy rates of about 60 % were obtained in beef cattle (Roy and Twargiramungu, 1996). In dairy cows, pregnancy rates of 40 to 55% were obtained after a FTAI following Ovsynch treatment (Pursley, 1997). Ovsynch protocol was capable of inducing a fertile ovulation in cyclic and anestrous beef cows and the pregnancy rates with FTAI were higher (59%) than those obtained with synchronized estrus (38%) using Syncromate-B (Geary *et al.*, 1998).

Mialot *et al.*, (1999) treated anestrous cows (between 60 -90 days postpartum) with GnRH-PGF<sub>2α</sub>-GnRH protocol with FTAI at day 10 (n=101) or insemination at observed estrus after 2<sup>nd</sup> GnRH injection (n=93). Out of 101 treated, 87 cows were inseminated at FTAI and 35 out of 87 (40.2%) cows conceived whereas 78 (64.2%) cows of 93 were inseminated at observed estrus and 50



out of 78 cows got finally conceived. In bovines, 100 % cows and 75 % heifers ovulated between 24 and 32 h after second GnRH injection (Pursley *et al.*, 1995). Fricke *et al.*, (1998). Postpartum interval to beginning of treatment, for synchronization of ovulation with FTAI, may also be important to obtain good conception rates. Pursley *et al.*, (1995) found higher conception rates at 60 days postpartum in animals treated with Ovsynch than control group.

As compared to cattle, very few reports are available in buffalo on synchronization of ovulation for FTAI (Baruselli *et al.*, 2003). Realizing the importance of such technique in buffaloes due to high incidence of silent estrus (Seren *et al.*, 1995), highly variable duration of estrus and consequent difficulty in predicting time of ovulation (Baruselli, 2001), recently some studies have been published on the use of Ovsynch technique in buffaloes (Berber *et al.*, 2002; Baruselli *et al.*, 2003; Neglia *et al.*, 2003; Paul and Prakash, 2005). Berber *et al.*, (2002) used two Ovsynch protocols with either GnRH or porcine LH on day 9 in post partum buffaloes at organized farm and observed similar ovulation rates (86.6% and 93.3%) after the two GnRH injections, irrespective of LH substitution. Baruselli *et al.*, (2003) treated 33 female buffaloes, 60 days postpartum, with different 1<sup>st</sup>GnRH dose of 10 or 20 µg and reported 60.6 % ovulations after first GnRH (Group I= 70.5 vs Group II = 50%) and 78.8 % buffaloes had synchronized ovulations after second GnRH injection. Animals synchronized during favourable reproductive season had better conception rates than unfavorable season (48.8 % vs 6.9%).

Khanna (2003) conducted a study on 45 postpartum buffaloes (35-45 days postpartum) and used conventional Ovsynch protocol (Day 0 – 1<sup>st</sup> GnRH (100 µg), Day 7 – PGF<sub>2α</sub> (25 mg) and Day 9- 2<sup>nd</sup> GnRH (100 µg)

in two groups of animals. In Group 1, FTAI was done after 12 and 24 h of 2<sup>nd</sup> GnRH whereas in Group 2, A.I. was done at detected estrus. The overall pregnancy rates were higher in Group 2 (60%) than Group 1 (40.1%). Number of services per conception were higher (P<0.05) in Group 1 (1.75±0.17) than Group 2(1.33±0.25)

Neglia *et al.*, (2003) compared two estrus synchronization protocols in Italian Mediterranean buffaloes, Group A (n=111) received 100 µg GnRH on Day 0, 375 µg PGF<sub>2α</sub> on day 7 and 100g GnRH on day 9 (Ovsynch); Group B (n=117) received an intravaginal drug release device (PRID) containing 1.55 g progesterone and a capsule with 10 mg estradiol benzoate for 10 days plus a luteolytic dose of PGF<sub>2α</sub> and 1000IU PMSG at PRID withdrawal. They found no difference in overall pregnancy rates between the two protocols (Group A, 36%; Group B, 28.2%).

Paul and Prakash (2005) evaluated efficacy of Ovsynch protocol for synchronization of ovulation and FTAI in Murrah buffaloes. They conducted two experiments, in experiment 1, 10 non-lactating cycling buffaloes received 10g GnRH analogue (Buserelin acetate) without regard to stage of the estrous cycle (day of treatment, day=0), followed by 25 mg PGF<sub>2α</sub> 7 days after first GnRH. A second GnRH injection (10 µg) was given 48 h after PGF<sub>2α</sub>. In experiment 2, 15 lactating, cycling buffaloes were subjected to the same Ovsynch protocol with FTAI 12 and 24 h after 2<sup>nd</sup> GnRH treatment. As controls, 75 lactating buffaloes were inseminated 12 h after detection of spontaneous estrus. In experiment 1, it was observed that ovulation occurred in 9/10 (90%) after 2<sup>nd</sup> GnRH treatment. In experiment 2, it was observed that pregnancy rates were 33 % for FTAI and 30.7 % for buffaloes inseminated following spontaneous

estrus, concluding that Ovsynch protocol can effectively synchronize ovulations in Murrah buffaloes. Ali and Fahmy (2007) recorded 62.5% and 100% ovulation following first and second GnRH treatments respectively with 37.5% conception in non -cyclic buffaloes.

### **Use of eCG-GnRH- PGF<sub>2α</sub>-GnRH (Ovsynch-Plus)**

Recently a modified protocol (Ovsynch-Plus) has been tried for induction of synchronized estrus and FTAI in Murrah buffalo heifers (Sharma *et al.*, 2004 and Singh *et al.*, 2004). Sharma *et al.*, (2004) compared the standard Ovsynch protocol and modified protocol (Ovsynch-Plus) in Murrah heifers, Group A (n=12) received standard Ovsynch treatment consisting of 10 µg GnRH on day 0 (start of treatment), 25 mg PGF<sub>2α</sub> on day 7 and second injection of 10µg GnRH on day 9 whereas Group B (Ovsynch-Plus) heifers (n=9) received similar Ovsynch treatment as in group A except that it was preceded by an additional injection of 600 IU eCG, two days prior to start of the Ovsynch treatment.

They observed overall pregnancy rate of 16.7 % (2/12) in Group A, which was considerably improved to 66.7% (6/9) in Group B. Similarly, Singh *et al.*, (2004) also studied the effect of modified protocol of Ovsynch-Plus in buffalo heifers and observed that in Group A (n=7) in which standard Ovsynch protocol was followed, none of the heifers responded to 2<sup>nd</sup> GnRH whereas in Group B (n=14) in which Ovsynch-Plus protocol was followed, 7/14 (50%) heifers became pregnant after FTAI. It was clearly indicated that Ovsynch – Plus protocol is comparatively better than Ovsynch alone and it is effective for inducing synchronized behavioral estrus in anestrus heifers with good fertility to fixed time artificial insemination. Ravikumar *et al.*, (2009) observed ovulation and first service conception rate as 100% vs 83.33% and

41.66% vs 33.33%, respectively in Ovsynch+CIDR vs Ovsynch treatment groups, respectively.

### **Other non-hormonal treatments**

Biostimulation describes the stimulatory effect of a male on estrus and ovulation through genital stimulation and priming pheromones (Chenoweth, 1983). It was suggested that the presence of male can hasten the onset of puberty (Izard, 1983), which is well documented in sheep and goats (Shelton, 1960). This male effect appears to be olfactory, involving the vomero-nasal organ having neural connections with the hypothalamus and this mediates the effects of priming hormones on ovarian function (Izard, 1983). Exposure of postpartum cows to bulls may shorten the duration of postpartum anestrus and/or increase ovulation (Fernandez *et al.*, 1993) but results are inconsistent. Exposure of postpartum cows to a vasectomized bull for 3-4 h twice daily resulted into cows conceiving from fertile matings earlier than control cows that were not exposed to teaser (Izard, 1983).

Economic benefits of using biostimulation to enhance induction and synchronization of estrus, reduction of postpartum anestrus, reduction in silent heat and improving ovulation rate may serve as an effective management tool (Singh, 2003). Postpartum anestrus is coupled to early lactating period, when most females, particularly buffaloes, are suckling. Herbal preparations like Janova, Quick Heat, Prajna etc. have been used in field practice with varying degree of success.

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